2950 Niles Road, St. Joseph, MI 49085-9659, USA
269.429.0300 fax 269.429.3852 hq@asabe.org www.asabe.org

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Effect of Treatment and Cultivar on the Ensiling of Corn Stover

R.E. Muck

USDA, ARS, US Dairy Forage Research Center, Madison, WI 53706

J.G. Coors

University of Wisconsin-Madison, Madison, WI 53706

T.L. Richard

Pennsylvania State University, University Park, PA 16802

M.P. Scott

USDA, ARS, Ames, IA 50011

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Abstract. *Nine cultivars of corn stover selected for ethanol potential were harvested (34 to 40% dry matter) and each ensiled with six treatments: untreated, lactic acid bacteria, cell-wall degrading enzymes, sulfuric acid, bacteria-enzyme combination and enzyme-acid combination. Ensiling was carried out in vacuum-sealed bags at ~22°C for 60 d. The untreated stovers ensiled well. Lactic acid bacteria and enzyme treatments had no effect on pH, but the bacteria-enzyme combination lowered pH in some cultivars. The acid and acid-enzyme treatments had low pH values ranging from 1.3 to 1.5. Lactic acid was generally highest in the bacteria-enzyme treatment whereas acetic acid was highest for the acid treatments. The acid treatments substantially reduced hemicellulose. Potential ethanol yield on average was highest in the bacteria-enzyme treatments.*

Keywords. Silage, Corn Stover, Lactic Acid Bacteria, Enzyme, Acid

Introduction

Corn stover is a potential lignocellulosic feedstock for ethanol production in the U.S. Last year in the U.S., over 35 million ha of corn were harvested (NASS, 2008). The amount of stover available for harvest should be approximately 9 Mg/ha. Even if half or more of the stover is left in the field to reduce erosion and help maintain soil organic matter, considerable quantities of stover are available for conversion into ethanol.

A key issue in developing such an industry based on stover is preservation of the stover between harvest and processing at a bioconversion facility. One potential for storage is ensiling. Ensiling the stover on farms is attractive for several reasons. First, storage of the stover would be distributed across many farms until needed at the processing plant. Second, ensiling should permit preservation of the stover across a wide range of moisture or dry matter (DM) contents. Third, the products of ensiling should help to stabilize stover and minimize spoilage between the farm and utilization at the processing plant. Finally, ensiling could potentially provide an opportunity for pretreating the stover, possibly reducing downstream processing costs.

Recent work suggests that stover is ensilable. Corn stover was ensiled at field-scale in a bag silo and in wrapped round bales with low losses (Shinners and Binversie, 2004). Ren et al. (2006) reported on ensiling stover with and without cell-wall degrading enzymes in mini-silos and at pilot-scale. Stover was ensiled successfully with and without various lactic acid bacterial inoculants over the range of likely DM contents (Muck and Shinners, 2006). These results indicate that stover can be ensiled, providing good preservation.

While stover can be ensiled successfully, the potential for pretreatment during silage storage has not been fully studied. Additionally, it is uncertain whether various pretreatments will act similarly across a range of stover cultivars. Thus the objectives of this study were to 1) compare the effectiveness of various silage additives on stover quality and 2) determine if the treatment effects were consistent across a range of corn cultivars.

Materials and Methods

Nine corn cultivars were harvested from plots at the West Madison Agricultural Research Station, Madison, WI in November 2006. A listing of the cultivars is in Table 1. These nine cultivars were selected from 50 tested the previous year, and the nine represent a range from the best to the poorest for ethanol potential from the stover. Ears were removed from all hybrids by hand. Then the stalks and leaves were cut and chopped. So the stover in this study consisted only of stalks and leaves, not cob nor husk. After harvest, the stover was bagged in plastic bags and frozen at -20°C until ensiling was performed.

All hybrids were analyzed for DM content by forced-air oven at 104°C overnight prior to ensiling. The DM contents ranged from 340 to 400 g/kg. Because of the similarity in DM content across the nine cultivars, treatments were set to the same wet-basis rates across the cultivars. Approximately 200 g stover was ensiled in 20 x 30 cm vacuum-sealed bags. Each cultivar was ensiled with six treatments: untreated control, standard inoculant (*Lactobacillus plantarum*, Ecosyl MTD/1, at 10^6 colony-form units (CFU)/g stover), enzyme (Multifect A40 at approximately 5 IU/g stover DM, Genencor Int'l, Rochester, NY), sulfuric acid (3% w/w stover), inoculant-enzyme combination, and acid-enzyme combination. Inoculants were prepared so that 1 g inoculant solution was sprayed on 100 g stover. The enzyme was diluted in order to apply at 1 g enzyme solution/100 g stover. Concentrated sulfuric acid was applied at 3 g/100 g stover. All treatments except the acid-enzyme treatment received additional water so that each treatment had an equivalent amount of water plus treatment across all 6 treatments. Three bags were

ensiled of each treatment per cultivar. Thus there were 18 bags per cultivar. After vacuum-sealing the bags, each bags was vacuum-sealed in a second bag to help insure good anaerobic conditions. Bags were stored at room temperature (~22°C) for 60 d. At 60 d, the silages were frozen at -20°C until they could be analyzed.

Three initial samples of each hybrid were taken at ensiling. These samples were analyzed for DM, pH, and lactic acid bacteria (Rogosa agar). The inoculant was analyzed for lactic acid bacteria. The ensiled stover was analyzed for DM, pH, fermentation products (Muck and Dickerson, 1988), fiber fractions [neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and lignin (Ankom Technology Corp., Fairport, NY)] and ethanol production potential via simultaneous saccharification and catabolism (SSC), an assay that measures the availability of sugars following a dilute acid pretreatment and treatment with commercial enzymes suitable for production of ethanol from lignocellulosic biomass (Haney et al., 2007).

Effects of treatment and cultivar were tested using the GLM procedure of SAS (SAS, 2001), using treatment, cultivar and their interaction as fixed effects. For the fiber analyses and SSC, batch was also used as a fixed effect in the statistical model. The LSMEANS statement was used to separate treatments and cultivars that were different from each other. Statistical significance was declared for $P < 0.05$.

Table 1. Cultivars ensiled, their initial characteristics, and their ethanol potential from 2005 trials based on in vitro ruminal fermentation (IVR) and simultaneous saccharification and catabolism (SSC).

Cultivar No.	Cultivar	DM, g/kg	pH	Lactic Acid Bacteria, log_{10}(cfg/g)	2005 IVR, mL/g	2005 SSC, f x 1000/g
1	W64A X A619	342	6.13	6.74	235	63.7
2	W64A X A619 bm2	358	6.02	6.18	252	128.3
3	W64A X A619 bm3	347	5.99	7.50	276	122.1
4	WQS C3 Syn2	370	6.17	5.70	271	81.7
5	W604S X LH244	359	5.94	6.11	256	67.2
6	W605S X TR7245	358	5.87	6.93	251	88.8
7	LH227 X LH279	383	5.87	6.82	249	61.3
8	P34M93	401	6.13	7.57	259	74.8
9	DK5143	347	6.52	6.75	244	57.7

Results and Discussion

The initial characteristics of the stovers at ensiling are shown in Table 1. All of the stovers had a relatively narrow range of DM contents, ranging from 342 to 401 g/kg stover. Most of the stovers had a pH within 0.2 units of 6.0 with the exception of cultivar 9. All of the stovers had high levels of lactic acid bacteria. The inoculant was applied at 5.00 log_{10}(cfu/g stover) so the natural lactic acid bacteria populations were 5 to 370 times greater than the inoculant population.

The pH values for the ensiled stovers are in Table 2. Treatment and cultivar were highly significant, and the cultivar*treatment interaction was significant at $P<0.04$. Across all cultivars, the pH of the inoculants and enzyme treatments were not significantly different from the untreated control. However, the combination of enzyme and inoculants did reduce pH relative to the control across all cultivars, but not significantly ($P < 0.05$) for some cultivars. Both treatments with sulfuric acid had pH value near 1.40. Cultivars 1-3 and 5 had the lowest pH values whereas the highest occurred with cultivars 8 and 9.

Table 2. The pH of ensiled stovers.

Cultivar	Control	Inoculant	Enzyme	Acid	Enz+Inoc	Enz+Acid	Average
1	3.88	3.85	3.87	1.35	3.85	1.38	3.03
2	3.83	3.80	3.85	1.38	3.83	1.37	3.01
3	3.84	3.90	3.89	1.38	3.88	1.40	3.05
4	3.97	3.95	3.99	1.45	3.92	1.45	3.12
5	3.87	3.89	3.88	1.34	3.84	1.34	3.02
6	3.95	3.89	3.92	1.42	3.86	1.45	3.08
7	3.93	3.93	3.92	1.40	3.88	1.54	3.10
8	4.00	3.99	4.02	1.45	3.97	1.47	3.15
9	4.04	4.05	4.06	1.46	3.95	1.45	3.17
Average	3.92	3.92	3.93	1.40	3.89	1.43	

LSD at $P<0.05$ = 0.070 for cultivar * treatment, 0.023 for treatment and 0.029 for cultivar.

Table 3. Lactic acid concentration (g/kg DM) of the ensiled stovers.

Cultivar	Control	Inoculant	Enzyme	Acid	Enz+Inoc	Enz+Acid	Average
1	66.0	71.9	71.3	0.8	74.2	1.1	47.6
2	73.8	81.7	75.6	0.2	81.0	0.1	52.1
3	69.3	72.3	74.9	1.1	75.2	1.1	49.0
4	63.0	64.2	61.3	0.6	67.8	0.5	42.9
5	48.0	64.5	68.2	0.9	63.8	0.9	41.1
6	54.5	55.7	58.0	1.1	55.9	0.8	37.7
7	50.1	53.0	58.4	0.7	60.2	0.9	37.2
8	49.7	55.3	60.6	0.8	58.5	0.8	37.6
9	65.6	63.6	58.6	1.2	85.3	1.4	45.9
Average	60.0	64.7	65.2	0.8	69.1	0.9	

LSD at $P<0.05$ = 6.56 for cultivar * treatment, 2.19 for treatment and 2.68 for cultivar.

Lactic acid concentrations were significantly affected by cultivar, treatment and their interaction (Table 3). Across cultivars, the enzyme plus inoculant treatment produced the most lactic acid; the inoculant and enzyme treatments were similar but less than the enzyme plus inoculant and greater than the untreated control. However, such trends were not consistent in all cultivars. Lactic acid concentrations were consistently low for the acid treatments, near the limit of detection. The highest concentrations were in cultivars 1-3 and lowest in cultivars 6-8.

Table 4. Acetic acid concentration (g/kg DM) of the ensiled stovers.

Cultivar	Control	Inoculant	Enzyme	Acid	Enz+Inoc	Enz+Acid	Average
1	23.1	22.9	25.3	23.2	22.9	25.0	23.7
2	20.3	15.4	21.2	20.8	16.3	22.1	19.4
3	19.9	20.2	21.2	22.0	20.0	21.1	20.7
4	19.8	13.8	19.5	21.9	15.9	21.1	18.7
5	15.6	19.2	21.6	23.8	20.3	22.0	20.4
6	21.6	16.5	21.2	21.7	16.5	20.7	19.7
7	18.5	14.0	19.2	27.0	15.6	27.1	20.2
8	16.5	15.8	21.0	29.0	16.6	26.7	20.9
9	21.5	16.7	16.8	26.5	14.3	24.7	20.1
Average	19.7	17.2	20.8	24.0	17.6	23.4	

LSD at $P<0.05$ = 2.59 for cultivar * treatment, 0.86 for treatment and 1.06 for cultivar.

Similar to lactic acid, acetic acid was significantly affected by cultivar, treatment and their interaction (Table 4). On average the treatments containing inoculant had the lowest acetic acid levels whereas the treatments receiving acid were the highest. However, those trends were not consistent across cultivars. One would expect the homofermentative inoculant to reduce acetic acid (Kung et al., 2003) relative to an untreated silage. Differences between cultivars, while statistically significant, are most likely not of practical significance given that the difference between the highest and lowest cultivar was only 5.0 g/kg DM.

Significant differences were observed in ethanol concentration (Table 5). On average, the highest ethanol concentrations occurred in the enzyme plus inoculant treatment followed by the enzyme treatment and the inoculant treatment, all of which had significantly more ethanol than the control. The treatments receiving acid had ethanol concentration near the detection limit. Two cultivars, 2 and 6, had much higher ethanol concentrations than the other cultivars. The cultivar by treatment interaction was statistically significant because the order of ethanol concentrations in the inoculant, enzyme and enzyme plus inoculant treatments were not consistent across all cultivars.

Both NDF and ADF were affected by cultivar, treatment and their interaction and are shown in Tables 6 and 7 respectively. The inoculant had no effect on NDF or ADF relative to the control. This would be expected because the bacterial strain in the inoculant produces no cell-wall degrading enzymes. In contrast, the enzyme does contain a mixture of cellulases and hemicellulases. Both enzyme-containing treatments reduced ADF and NDF relative to the control across all cultivars. In several cases, the differences within a cultivar were not quite

significant, one cause of the significant interaction effects. The acid-containing treatments produced large reductions in NDF, but the reduction in ADF compared to the control was similar to the effect of enzyme treatment. Cultivars 1-3 and 6 had the lowest fiber concentrations whereas cultivars 7-9 had the highest concentrations.

Table 5. Ethanol concentration (g/kg DM) of the ensiled stovers.

Cultivar	Control	Inoculant	Enzyme	Acid	Enz+Inoc	Enz+Acid	Average
1	2.5	4.1	7.4	0.9	15.3	0.6	5.1
2	17.3	30.8	27.6	0.7	41.5	0.5	19.7
3	9.4	12.2	18.2	1.0	29.7	1.0	11.9
4	8.1	10.5	17.2	0.7	27.5	0.6	10.8
5	3.5	5.5	16.4	0.9	15.6	0.9	7.1
6	23.6	27.7	30.6	1.4	37.8	1.7	20.5
7	3.1	2.7	6.0	0.5	10.7	0.1	3.8
8	1.5	1.9	3.7	0.4	7.8	0.7	2.7
9	1.6	1.6	1.5	0.0	2.5	0.4	1.3
Average	7.8	10.8	14.3	0.7	20.9	0.7	

LSD at $P<0.05$ = 4.23 for cultivar * treatment, 1.41 for treatment and 1.73 for cultivar.

Table 6. Neutral detergent fiber concentration (g/kg DM) of the ensiled stovers.

Cultivar	Control	Inoculant	Enzyme	Acid	Enz+Inoc	Enz+Acid	Average
1	596	585	578	391	564	425	523
2	594	610	562	386	573	391	519
3	568	571	524	357	538	348	484
4	606	621	578	437	583	409	539
5	618	622	589	423	592	400	541
6	594	604	576	406	562	400	524
7	637	636	611	473	619	475	575
8	655	650	614	480	600	473	579
9	666	659	623	492	615	482	590
Average	615	618	584	427	583	423	

LSD at $P<0.05$ = 25.2 for cultivar * treatment, 8.4 for treatment and 10.4 for cultivar.

The difference between NDF and ADF is an estimate of the amount of hemicellulose in the stover. As with NDF and ADF, hemicellulose was affected by cultivar, treatment and their interaction (Table 8). The inoculant had no effect on hemicellulose concentration relative to the

control. The enzyme-containing treatments produced a small but statistically significant reduction in hemicellulose on average, but in some cultivars the hemicellulose concentrations in these treatments were not different from their respective controls, the principal cause of the interaction term being significant. The acid-containing treatments consistently produced large reductions, approximately 60%, in the amount of hemicellulose.

Table 7. Acid detergent fiber concentration (g/kg DM) of the ensiled stovers.

Cultivar	Control	Inoculant	Enzyme	Acid	Enz+Inoc	Enz+Acid	Average
1	337	331	317	295	307	322	318
2	341	351	318	294	322	297	320
3	321	324	285	275	293	270	295
4	342	351	322	329	324	310	330
5	353	362	325	324	328	305	333
6	342	344	323	305	311	299	321
7	376	374	354	364	362	367	366
8	381	387	357	365	344	364	366
9	389	388	353	373	352	367	371
Average	354	357	328	325	327	322	

LSD at $P<0.05$ = 20.2 for cultivar * treatment, 6.7 for treatment and 8.3 for cultivar.

Table 8. Hemicellulose concentration (g/kg DM) of the ensiled stovers.

Cultivar	Control	Inoculant	Enzyme	Acid	Enz+Inoc	Enz+Acid	Average
1	260	254	262	96	257	103	205
2	253	259	244	93	250	93	199
3	247	247	239	82	245	78	189
4	264	270	256	107	259	98	209
5	265	259	264	99	263	95	208
6	251	261	253	101	251	101	203
7	262	262	257	109	256	108	209
8	273	264	257	114	255	110	212
9	277	271	270	119	263	115	219
Average	261	261	256	102	256	100	

LSD at $P<0.05$ = 9.2 for cultivar * treatment, 3.1 for treatment and 3.8 for cultivar.

For the other fiber fractions, there were no significant interactions between cultivar and treatment. The results averaged by treatment and cultivar are shown in Table 9. Only the acid

treatments reduced ADL relative to the control. However, lignin concentration (essentially ADL minus ash) showed no effect due to treatment. This suggests that the acid treatments solubilized some ash in the cell wall. Cellulose concentrations were similar for the inoculant and control treatments. All enzyme-containing and acid-containing treatments produced similar reductions in cellulose relative to the control, approximately 30 g/kg DM.

The availability of sugars following dilute acid and enzymatic digestion as measured by SSC is shown in Table 9. There was no significant interaction between cultivar and treatment. The only treatment to have a higher SSC value than the control was the enzyme plus inoculant treatment ($P<0.06$). The acid treatments very significantly reduced the SSC value. This is the opposite of what we expected. The reason for this is under investigation. It is likely the high acid load in these samples may have adversely affected the assay.

Table 9. Acid detergent lignin, cellulose and lignin concentrations (g/kg DM) and estimate of the sugar available for ethanol production (SSC, rel. fluorescence units/g) of the ensiled stovers.

	Acid Detergent Lignin	Cellulose	Lignin	SSC
Treatment				
Control	33	321	19	36000
Inoculant	34	322	21	37800
Enzyme	34	294	20	34200
Acid	29	296	20	21700
Enz+Inoc	36	291	20	38400
Enz+Acid	30	292	21	22100
LSD at $P<0.05$	2.3	6.3	2.4	2380
Cultivar				
1	33	285	22	32500
2	40	281	16	33300
3	29	265	10	35300
4	27	303	18	32200
5	29	304	19	30600
6	32	288	21	32500
7	36	330	27	29200
8	37	329	25	30200
9	32	338	22	29600
LSD at $P<0.05$	2.8	7.8	3.0	2910

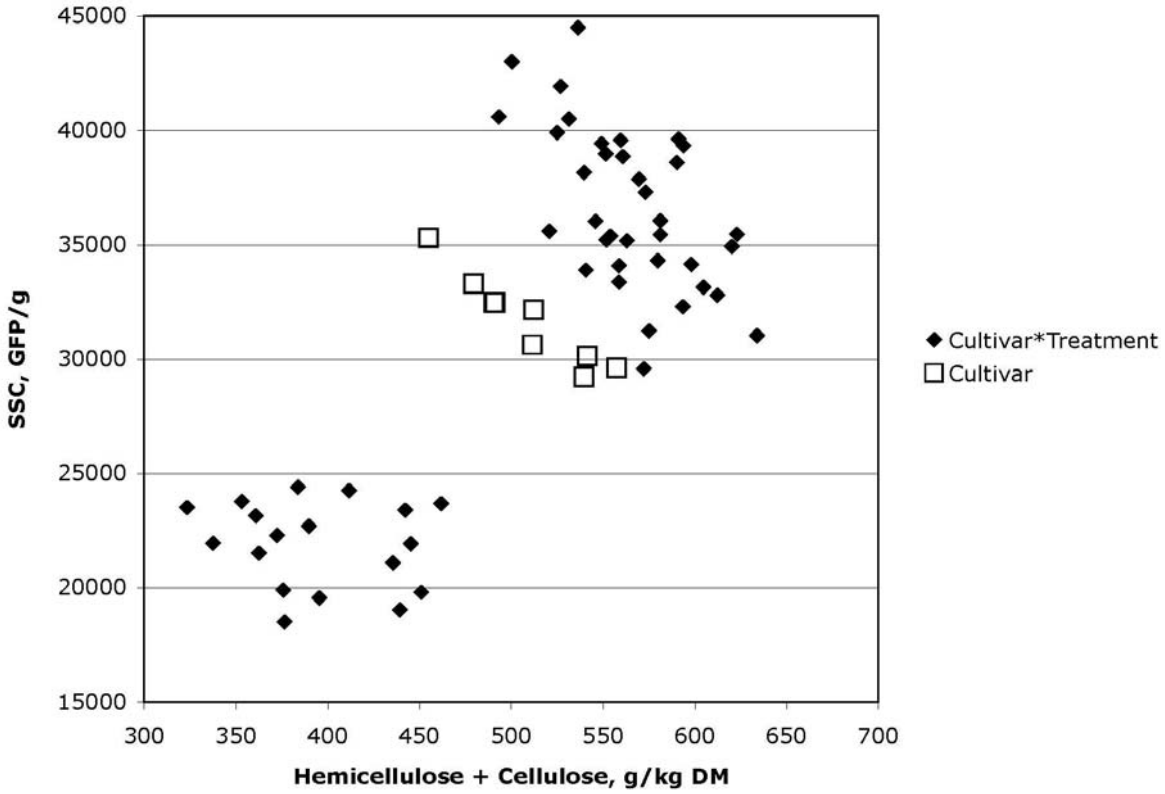

Figure 1. Relative, estimated sugars available for ethanol production (SSC) in ensiled stover as correlated with hemicellulose plus cellulose concentration.

The SSC values varied by cultivar. The two highest were cultivars 2 and 3, cultivars that contain a brown midrib gene. The lowest were cultivars 5, 7, 8 and 9. Interestingly the rankings are similar to the previous year where 2 and 3 were the highest and 1, 5, 7, and 9 were the lowest (Table 1) even though there are substantial differences in the magnitudes between years. Plotting all of the cultivar by treatment means for SSC value against the structural carbohydrates (hemicellulose plus cellulose), one observes that all of the acid-treated ensiled stovers form a cloud in the lower left portion of the graph (Fig. 1). This result should be treated with caution due to the afore-mentioned possibility of interference of the acid treatments with the assay. However, the rest of the conditions exhibit a negative correlation between SSC value and the amount of structural carbohydrate. This is also observed when plotting the average cultivar values (Fig. 1). This relationship illustrates the importance of considering the accessibility of structural carbohydrates to enzymes used for ethanol production, in addition to the content of structural carbohydrates.

Conclusions

Nine cultivars of corn stover representing a diverse set relative to ethanol potential were treated with six treatments (control, homofermentative inoculant, cell-walling degrading enzymes, sulfuric acid, enzyme-inoculant combination, enzyme-acid combination). While there were some statistically significant cultivar by treatment interactions, these interactions were of little practical significance. In general, the cultivars ranked similarly in ethanol potential compared to the previous year.

The inoculant treatment had no significant effects relative to the control treatment except for shifting silage fermentation: increasing lactic acid and ethanol, decreasing acetic acid. The cell-wall degrading enzymes alone had no effect on pH but increased the concentrations of all the major silage fermentation products. The primary effect of the enzymes on the cell walls was in hydrolyzing cellulose (~30g/kg DM). The enzyme plus inoculant treatment performed similar to the enzyme treatment except it reduced acetic acid and increased ethanol potential relative to the control. The acid and enzyme plus acid treatments acted similarly, reducing pH to approximately 1.40 and minimizing fermentation products except for acetic acid. These treatments reduced hemicellulose content by approximately 60% relative to the control and reduced cellulose similarly to the enzyme treatment. Ethanol potential was substantially reduced by the acid treatments, but this was most likely due to problems created by the acid on the assay. Until this issue is resolved, we conclude that the treatment that most likely improved ethanol potential was the enzyme-inoculant combination.

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